SPERMATOZOAN ULTRASTRUCTURE AND ITS BEARING ON GASTROPOD CLASSIFICATION AND EVOLUTION*

John M. Healy**

THE study of molluscan classification has progressed a long way since the adoption of the Linnaean system of binominal nomenclature for all zoological (and botanical) names. Early preoccupation with shell morphology has now been tempered by a recognition of the importance of soft-part anatomy and radular teeth structure, though it should be added, shells remain an extremely useful indicator of genetic relationship (for example, the study of fossil molluscs relies almost entirely on shell structure). General anatomy and shell structure/colour forms the basis of modern taxonomic and evolutionary malacology, but increasingly with the assistance of more sophisticated techniques of analysis such as electron microscopy, electrophoresis (protein analysis) and genetic analysis (chromosome, DNA, RNA research). My work, since 1979, has been directed towards comparative spermatozoan morphology in the Mollusca, particularly Gastropoda, using transmission electron microscopy (TEM) — the goal of this research being to attempt to unravel evolutionary relationships within the Gastropoda (and other molluscan classes) and also to determine the correct systematic placement of numerous "problem" families or superfamilies. The "wet" collection of molluscs held in the Australian Museum (Sydney) has been of considerable help to the progress of this research, since it contains well preserved animals of important, rare or deep-sea species otherwise unavailable for study.

TEM requires first the fixation (chemical preservation) of tissues to be examined (usually with glutaraldehyde or formalin but never alcohol), then treatment of this tissue with a heavy-metal stain/fixative (usually 1% osmium tetroxide), then dehydration in alcohol (20-100% stages) and embedding in a plastic resin (hardened in an oven). Ultrathin sections of plastic-embedded tissue are cut using an ultramicrotome, then these sections are collected on to a grid (3 mm diameter, usually made of copper and having very fine mesh to support sections). Subsequently the sections are stained with heavy-metal solutions (lead citrate, uranyl acetate), then viewed with the electron microscope.

TEM permits one to examine in fine detail the internal structure of any cell. The method is particularly well suited for spermatozoan morphology since these are small often complex cells which occur in great numbers, so that sections through many or all zones of the cell can be observed often in a single section of tissue. By piecing together information from different photographs the three-dimensional internal structure of a spermatozoon can be reconstructed.

Animal spermatozoa generally consist of a head, midpiece and tail (Figs 1, 5), though there are a number of exceptions to this, particularly among the invertebrates. The sperm head has two components — the acrosome (the organelle which first "reacts" with the egg membrane or coatings to permit union of sperm and egg genetic material) and the nucleus (containing the male genetic material or chromosomes in a condensed form). Posterior to the head is the midpiece. In this region of the spermatozoon, energy-producing organelles known as mitochondria are arranged around the structures responsible for sperm motility — the centrioles and axoneme (the axoneme forming the major constituent of the tail). The morphology of the midpiece varies greatly from simple (Fig. 5) to complex (Figs 7, 8).

My own studies, and those of a number of other workers, have shown that in the gastropod subclass Prosobranchia (most of the shelled, marine snails), members of the order Archaeogastropoda have the simplest spermatozoa (see Figs 1, 5). The Archaeogastropoda includes superfamilies such as the Pleurotomariacea (e.g., the deep-sea slit shell

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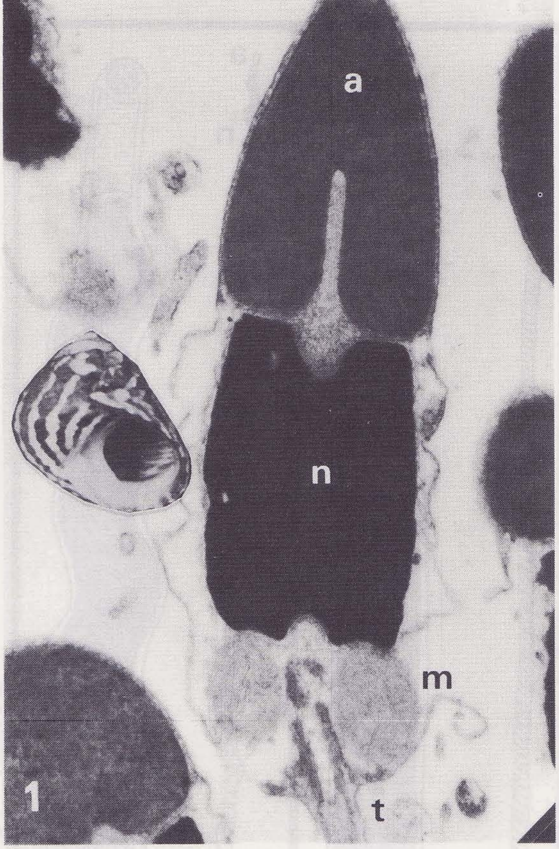
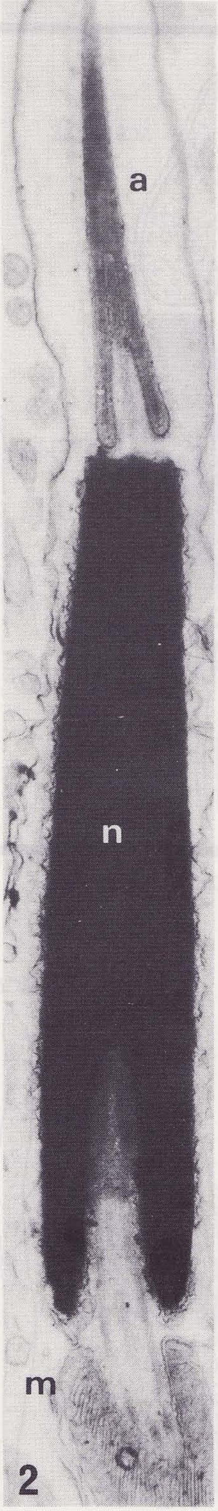
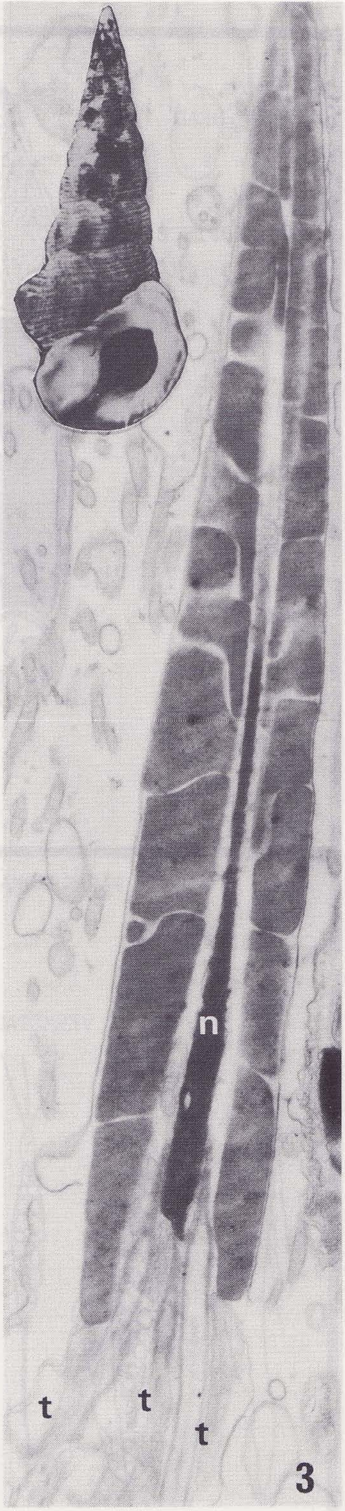






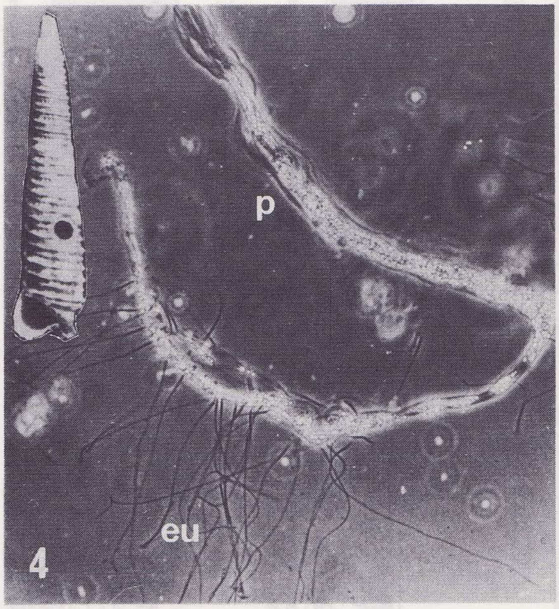
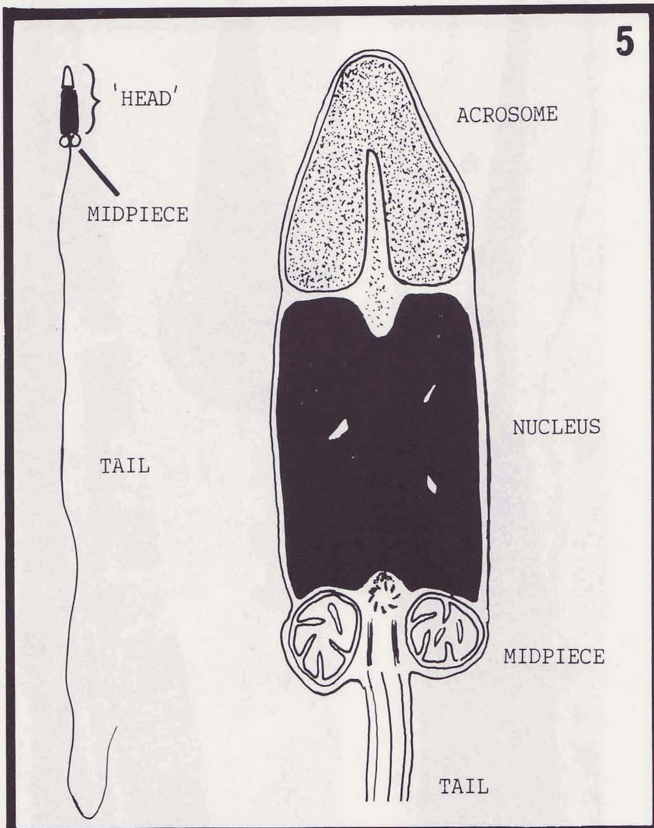
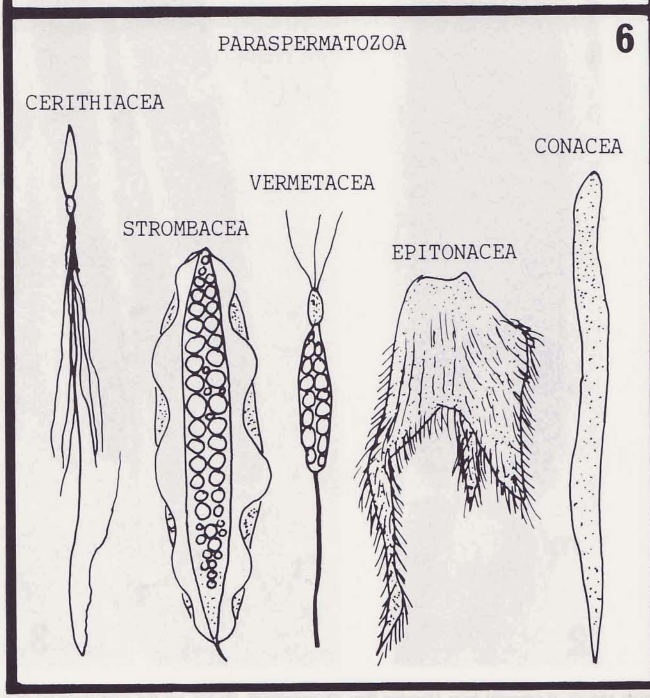
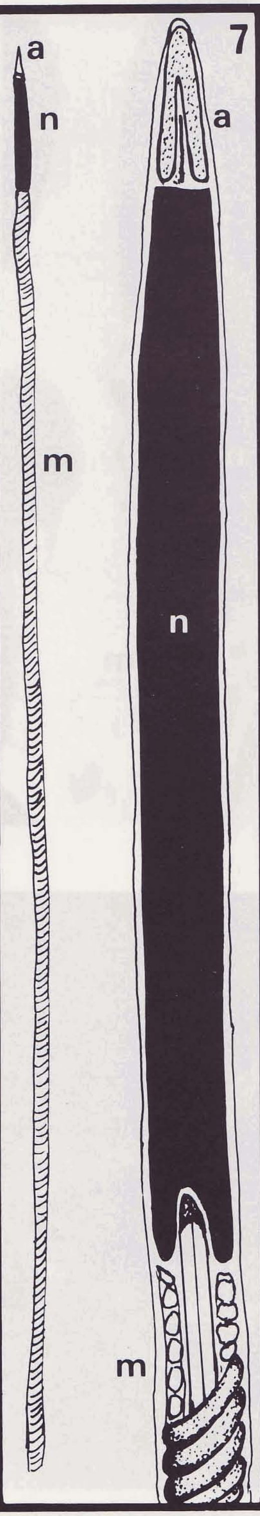


Fig. 1. *Spermatozoa of an archaeogastropod, the striped top shell (Austrocochlea obtusa, (Lamarck) from Moreton Bay, Queensland). Visible are the acrosome (a) and nucleus (n) of the "head", the midpiece (m) and a portion of the sperm tail (t). (mag. ×25 000; shell ×1).* Fig. 2. *Euspermatozoa (fertile sperm) of a mesogastropod, the mud whelk* Pyrazus ebeninus *Bruguiere from Moreton Bay. The acrosome (a) and nucleus (n) of the head are shown, together with the initial portion of the midpiece (m) (mag. ×38 000).* Fig. 3. *Paraspermatozoon (non-fertile sperm) of* Pyrazus *— showing nuclear remnant (n) of the head, and multiple tails (t). Sperm dimorphism occurs in many higher prosobranch gastropods, with paraspermatozoa possibly providing nutrition for euspermatozoa (mag. ×220 000; shell of* Pyrazus *×0.5).* Fig. 4. *In the mesogastropod superfamily Triphoracea, shells are usually sinistrally-coiled (*Viriola *sp. shell pictured ×2). These snails produce large paraspermatozoa (p) to which become attached clumps of euspermatozoa (eu, fertile sperm) — this combination being very mobile (mag. ×1 000).*







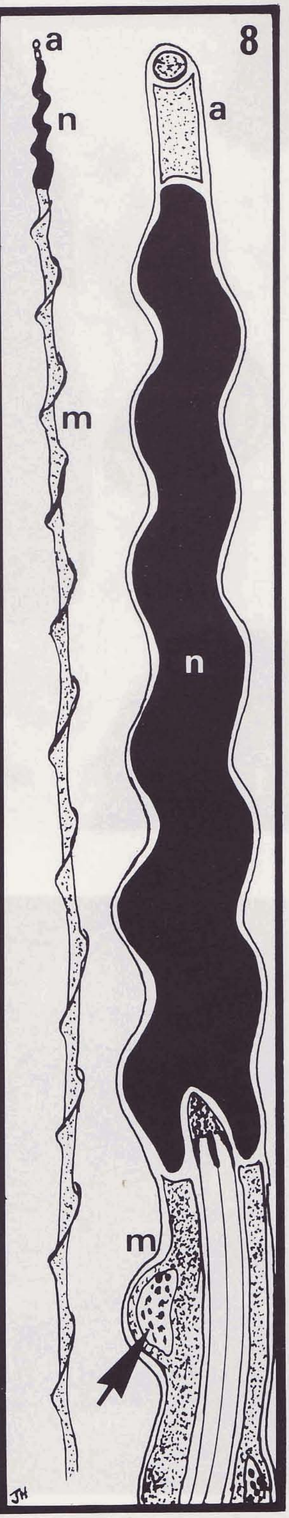
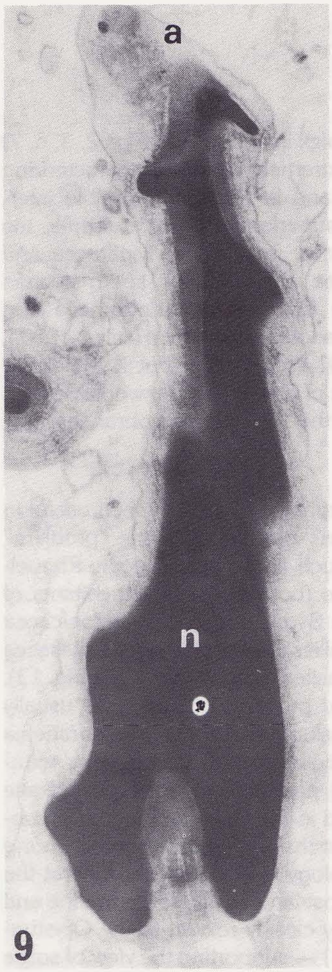
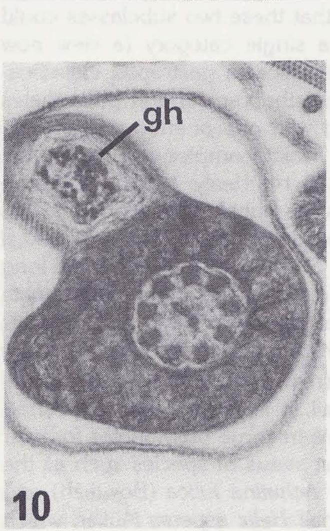
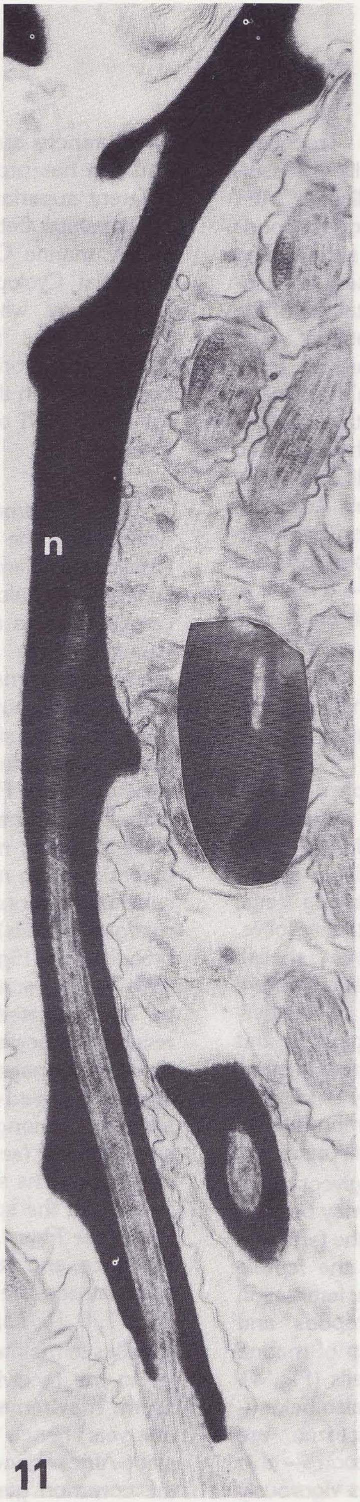
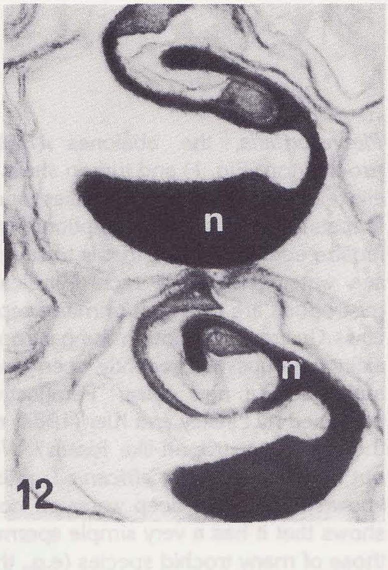


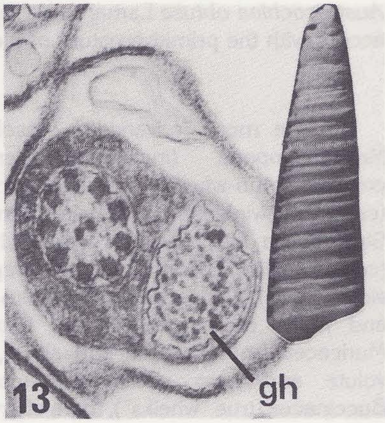
Fig. 5. *Spermaotozoa of a typical archaeogastropod — the top shell* Austrocochlea obtusa *(Lamarck): (left) whole cell (mag. ×2 000), (right) detail of acrosome, nucleus, midpiece (mitochondria surrounding centrioles) and proximal portion of tail (mag. ×25 000).* Fig. 6. *Paraspermatozoa (non-fertile sperm) of various meso- and neogastropods. These sperm are produced alongside the euspermatozoa (Fig. 7) in the gonad. In the Epitoniacea (second from right) euspermatozoa (shown here as "hairs") are attached in lage numbers to each paraspermatozoa (left to right mag. ×900, ×650, ×800, ×70, ×1 000 — figures redrawn and enlarged from Nishiwaki 1964 and Tochimoto 1967).* Fig. 7. Head (acrosome (a) plus nucleus (n)) and initial portion of the midpiece (m) of a mesogastropod euspermatozoon (mag. left ×2 000; right ×25 000). Fig. 8. *Head (acrosome (a), nucleus (n)) and initial portion of midpiece (m) of a generalized opisthobranch/pulmonate gastropod spermatozoon. Note the spirally-keeled nucleus and midpiece. Arrow indicates the glycogen-filled helix of the midpiece. (mag. left ×2 000; right ×25 000).*











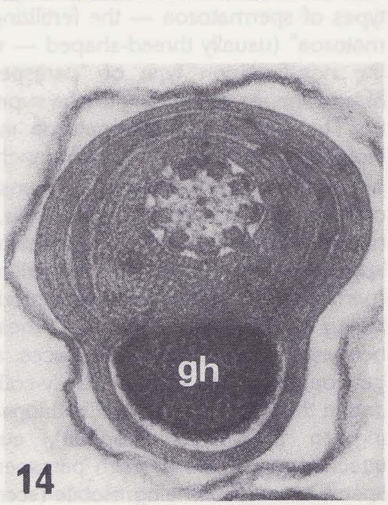


Fig. 9. *Head (acrosome (a) plus nucleus (n)) of a nudibranch (sea slug) spermatozoon* (Pteraeolidea ianthina, *Angas from Moreton Bay, Queenland) (mag. ×28 000).* Fig. 10. *Cross-section of the sperm midpiece of* Pteraeolidea *showing the glycogen-filled helix (gh) (mag. ×58 000).* Fig. 11. *Portion of the sperm nucleus of the bubble-shell* Tornatina *sp. (from Moreton Bay, Queensland: shell inset — ×10). Note the helical keels (mag. ×40 000).* Fig. 12. *Two cross-sectional views of the* Tornatina *sperm nucleus showing its complex helical keels (mag. ×20 000).* Fig. 13. *Cross-section of the midpiece region of a pyramidellid spermatozoon showing glycogen helix (gh)* (Cingulina *sp. from Moreton Bay, Queensland: shell inset ×2). Sperm morphology indicates that the Pyramidellidae belong to the opisthobranch/pulmonate group of gastropods and not the Prosobranchia (mg. ×58 000).* Fig. 14. *Sperm midpiece of the common garden snail* Helix aspersa Müller *(from Brisbane), showing glycogen helix (gh). Pulmonate gastropods (most air-breathing snails) are closely related to the opisthobranchs (sea slugs, bubble shells and allies), as evidenced by their sperm structure (mg. × 58 000).*

Pleurotomaria, the abalones (*Haliotis*)), Trochacea (trochid/top (Fig. 1) and turban shells and allies), Fissurellacea (keyhole limpets and allies) and Patellacea ("true" limpets). With some exceptions, archaeogastropods fertilize eggs externally, that is to say, in the surrounding sea water. The fossil record indicates that archaeogastropods are the most primitive representatives of the class Gastropoda, though the gastropod status of some extinct groups has recently been questioned by some authorities (a new class, "Paragastropoda" has been proposed by Linsley and Kier (1984) to contain many of the coiled, gastropod-like fossils). Work I have carried out on *Pleurotomaria africana* Tomlin (a slit-shell, now known to occur in deep water off north-west Australia) shows that it has a very simple spermatozoon similar to those of many trochid species (e.g., the striped top shell *Austrocochlea obtusa* Lamarch — Fig. 1) — a result in accord with the primitive nature of pleurotomariids.

In the more "advanced" prosobranch orders — Mesogastropoda (including: Cerithiacea (sand-creepers, mud-whelks and allied families), Littorinacea (common winkles), Vermetacea (true worm shells), Strombacea (strombs and spider shells), Cypraeacea (cowrie shells), Epitoniacea (wentletraps and the floating violet snails), Tonnacea (triton, helmet and tun shells) and many other groups) and neogastropoda (e.g., Muricacea (murex shells and allies), Volutacea (olive, volute and harp shells), Conacea (cone shells), Buccinacea (true "whelks"), there often exist two *different* types of spermatozoa — the fertilizing type of "euspermatozoa" (usually thread-shaped — see Figs 2, 7) and the non-fertilizing type of "paraspermatozoa" (many bizarre shapes, depending on the superfamily — see Fig. 6). The role of paraspermatozoa in the reproductive biology of these "higher" prosobranchs is still uncertain, but because of the substantial deposits of glycoprotein or lipoprotein contained in these cells, they may be connected with the nutrition of euspermatozoa (the fertilizing sperm) or even provision of nutrients for the female (perhaps as a contribution to egg production: fertilization taking place internally in all mesogastropods and neogastropods). In the Triphoracea, a group of marine gastropods chiefly with sinistrally-coiled shells (Fig. 4), and in the Epitoniacea and Cerithiopsacea (also belonging to the Mesogastropoda), euspermatozoa are attached in clumps to each paraspermatozoon — this sperm association being mobile (sometimes vigorously so) (see Fig. 4). This unusual arrangement may provide a means of sperm transference in these snails since males lack a copulatory apparatus, though some other snails overcome the same problem by transferring sperm packets or "spermatophores" to females. Both the euspermatozoa and paraspermatozoa of higher prosobranchs are complex in shape (see Figs 2, 3, 6, 7) and this has proved extremely useful in characterizing different superfamilies and in checking possible interrelationships between superfamilies. For example, the chiefly marine Cerithiacea, freshwater Viviparacea and terrestrial Cyclophoracea share smiliar spermatozoan morphology, while the Epitoniacea, Triphoracea and Cerithiopsacea form another group (Healy, 1983a, 1986). This work is still progressing, but it is helping considerably in the task of constructing a workable plan (or phylogeny) of how the "higher" prosobranchs may have evolved.

Spermatozoan morphology has also been useful in determining the subclass position of some "problem" families/superfamilies such as the microscopic Rissoellidae and Omalogyridae (both common inhabitants of coralline algaes around Sydney), the Architectonicacea (sundial shells and allies) and the Pyramidellacea (common external "parasites" of bivalves — see Fig. 13). These four groups (and probably others) have usually been considered as belonging to the Prosobranchia (within the order Mesogastropoda), but sperm ultrastructure (e.g., Fig. 13) and general anatomy indicate that all should be placed in the Opisthobranchia (a subclass including nudibranchs and bubble shells, among others). Sperm morphology also clearly shows that the subclass Pulmonata (most land snails, some marine and mangrove snails) is very closely related to the Opisthobranchia (see Figs 8, 14) — supporting the view of some 19th century scientists that these two subclasses could be amalgamated into a single category (a view now receiving renewed acceptance in gastropod classifications). Spermatoza of opisthobranchs and pulmonates are long, thread-shaped with one or more spiral keels and are among the most complex of all animal spermatozoa (see Figs 8-14; Healy 1983b). In some opisthobranchs such as the bubble-shell *Tornatina*, the nucleus of the sperm head may exhibit intricate helical keels (see Figs 11, 12). Spiral keels present in the long midpiece and tail region of opithobranch/pulmonate spermatozoa are formed largely of glycogen deposits (Figs 7, 8, 10, 13, 14) which may act, perhaps, as energy supplies to maintain sperm motility or viability after transfer to the receiving snail. It is known that transferred sperm may remain viable in some pulmonates for over one year, hence the pest status of species such as the giant African land snail *Achatina fulica* (Bowdich) and the common garden snail *Helix aspersa* Müller, which once fertilized, have enormous breeding potential. Paraspermatozoa are completely absent in the Opisthobranchia and Pulmonata, but fortunately (from a taxonomic point of view), the complexity of the fertilizing spermatozoa continues to provide insight into relationships within these two gastropod subclasses.

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The awards will be presented at a ceremony at the Society's Rooms (in the Education Centre, Taronga Zoo) at 4.30 p.m. on Tuesday, November 10 1987.

The guest speaker will be Dr Peter Pockley of the Public Affairs Unit at the University of New South Wales.